events (x2 = 25, p = 0.0001) and death (x^2 = 21, p = 0.001); while the presence of ischemia (x^2 = 11, p = 0.001) as the best predictor of soft events. The 4 year hard event free survival was 90% in pts with large defects and 97% in pts with no or small defects (p = 0.01). The 4 year freedom from revascularization was 78% in pts with ischemia and in pts with no ischemia (p = 0.05).

Thus, the predictors of hard and soft events are different in pts with stable CAD. The perfusion defect size, a marker of LV function, is an important predictor of death while ischemia is a more important predictor of other events.

941 Cardiovascular Molecular Biology: Functional Modifiers

Monday, March 17, 1997, Noon–2:00 p.m.
Anaheim Convention Center, Hall E
Presentation Hour: Noon–1:00 p.m.

MONDAY POSTER

941-140 Myocardial Prostaglandin Receptors (EP_3 Subtype) Inhibit the Inotropic Effect of β-Adrenergic Stimulation

T. Hohlfeld, K. Schrör. *Institut für Pharmakologie, Heinrich-Heine-Universität, Düsseldorf, Germany*

Prostaglandin receptors (EP_3 subtype) have recently been identified in the human heart. The functional role of these receptors, however, is unknown. In order to examine cardiac EP_3 receptor function, prostaglandin (PG) E_1 (1 nmol/min) was infused into the LAD of anesthetised open-chest minipigs. Heart rate, systolic blood pressure and regional contractile activity (sonomicrometric crystals, area supplied by the LAD) were not changed at baseline conditions. In contrast, PGE_1 markedly decreased the inotropic effect of 3 μg/min isoprenaline (i.v.) by 74 ± 18% (n = 5, p < 0.05 vs. control). Following pretreatment with reserpine (50 μg/kg, i.v., 8 h before surgery), PGE_1 was equally effective (85 ± 8% reduction of isoprenaline-stimulated contractility, n = 4, p < 0.05 vs. control), suggesting a postsynaptic effect of PGE_1. The EP_3 receptor-selective agonist M&B 28.767 (1 nmol/min, i.c.) also decreased the inotropic action of isoprenaline by a similar extent, while butaprost (5 nmol/min, i.c., selective for EP_2 receptors) did not. This indicates that myocardial EP_3 receptors mediate the observed negative inotropic action of PGE_1. The positive inotropic action of the phosphodiesterase inhibitor milrinone (0.2 mg/kg, i.v.), but not of ouabain (10 μg/kg, i.v.), was also inhibited by PGE_1, suggesting that myocardial EP_3 receptors selectively reduce cAMP-mediated contractile effects. This was confirmed by in vitro experiments, showing an inhibition of sarcolemmal membrane cAMP formation by PGE_1 (EC_{50} = 2.5 nM). It is concluded that myocardial EP_3 receptors inhibit cAMP-mediated inotropic effects. This provides a rational explanation of the well-established antiischemic action of endogenous and exogenous prostaglandins.

941-141 Pulmonary Artery Banding Upregulates Vascular Endothelial Growth Factor and its Flk1 Receptor in Pig Hearts

S. Kuki, E.P. Bauer, M. Arras, R. Zimmermann, W. Schaper. *Max-Planck-Institute, Bad Nauheim, Germany*

Although vascular endothelial growth factor (VEGF) is a potent and specific endothelial mitogen that is able to induce angiogenesis, little is known regarding the role of VEGF in *in vivo* cardiac hypertrophy which requires a proportional coronary artery growth. We investigated the gene expression of VEGF and its Flk1 and Flt1 receptors using right ventricular hypertrophy (RVH) after pulmonary artery banding (PAB) in pigs, which exhibit a coronary artery anatomy and distribution similar to humans. 35 pigs (5 groups) were used, each group included 4 banded (B) and 3 sham (S)-pigs. Pigs were killed at 2h, 1d, 3d, 7d, and 24d after PAB. RV/(IVS + LV) in weight was used for index of RVH. VEGF, Flk1, Flt1 and atrial natriuretic factor (ANF) mRNA were analyzed by Northern blot hybridization. Mean trans-banding pressure gradient was 29 mmHg. Significant RVH was observed within the first week after PAB. The expression of ANF in RV was transiently observed during the development of RVH, but not in the established RVH. The rapid induction of VEGF and the following persistent expression of Flk1 were observed prior to progression of RVH. In addition, we investigated transmural distribution of these mRNAs at 2h after PAB, however, there were no differences in mRNAs level between subendocardium and subepicardium. The expression of Flt1 could not be observed. The extremely rapid expression of the ligand and receptor mRNA before hypertrophy suggest functions of the VEGF-system in addition to angiogenesis.

	Time after PAB				
	2h	1d	3d	7d	24d
RV/(IVS + LV)	1.07	1.0	1.31	1.65**	1.63**
ANF/18S	1.12	2.42**	5.24**	1.63	1.14
VEGF/18S	2.03**	1.57	1.11	0.95	1.06
Flk1/18S	1.56*	2.53**	1.84**	1.04	1.18

Values (mean) for B are expressed as fold-increase over S. *p < 0.05, ** p < 0.01 vs S.

941-142 Decreased Levels of I_{KACh} mRNA in Patients with Chronic Atrial Fibrillation but no Changes in the Sarcoplasmic Reticulum Calcium ATPase and Phospholamban

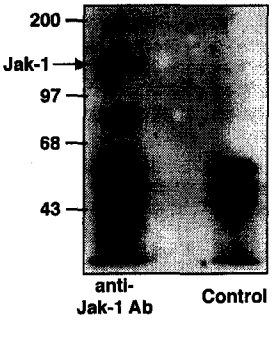
B.J. Brundel, I.C. Van Gelder, R.H. Henning, L.E. Deelman, A.E. Tuinenburg, W.H. Van Gilst, H.J. Crijns. *Dept. of Cardiology and Clinical Pharmacology, Thoraxcenter, University Hospital Groningen, The Netherlands*

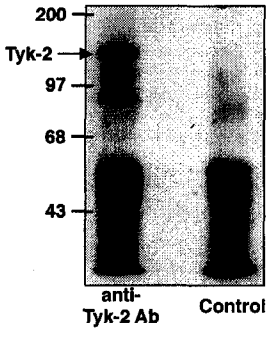
Impairment of atrial and ventricular function is an important consequence of atrial fibrillation (AF). This may be caused by a tachycardiomyopathy (TCM). No data on the underlying mechanism are available. The aim of this study is to investigate mRNA levels of proteins influencing the calcium and potassium handling, sarcoplasmic reticulum calcium ATPase (SERCA), phospholamban (PI) and one component of the I_{KACh}, the cardiac inward rectifier (CIR). Right atrial appendages (RAA) were obtained from 18 patients with AF and from 19 controls in sinus rhythm who were matched for age, sex, underlying disease, drugs and functional class. Previous duration of AF was 9 ± 3 months. Total RNA of RAA was isolated and reversely transcribed into cDNA. In a single semi-quantitative polymerase chain reaction the mRNA of interest and of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were coamplified and separated by gel-electrophoresis. cDNAs of the ethidium bromide stained gels were quantified by densitrometry. The CIR mRNA content was significantly reduced (−36%, p < 0.05). SERCA and PI mRNA levels were unchanged. GAPDH levels were identical for both groups. *Conclusion:* chronic AF did not affect mRNA levels of SERCA and PI. However, the mRNA level of one component of I_{KACh}, (CIR) was significantly downregulated in the RAA of patients suffering from chronic AF.

941-143 Involvement of Janus kinases (Jak) in Urokinase Receptor (uPAR) Signalling – A Novel Pathway to Alter Gene Expression in the Development of Atherosclerosis?

A. Weis, I. Dumler, R. Dietz, D.C. Gulba. *Humboldt University at Berlin, Berlin, Germany*

The binding of urokinase-type plasminogen activator (uPA) to cell surfaces via its specific receptor (uPAR) facilitates migration, invasion and matrix degradation for vascular smooth muscle cells (SMCs) and endothelial cells (ECs) – key events in atherosclerosis and in intimal thickening after vascular injury. However, the exact signalling pathways of the uPA/uPAR system are so far unknown. This study proposes a role for the Jak/STAT pathway in the uPAR-mediated signalling by providing a close association of the Janus kinases Jak1 and Tyk2 with the uPAR in human aortic SMCs and human umbilical vein ECs. Cell lysates of a primary culture of SMCs and ECs were immunoprecipitated with anti-uPAR monoclonal antibodies. Precipitated proteins were separated by gel electrophoresis, transferred to a PVDF membrane, immunoblotted with polyclonal anti-Jak1, anti-Jak2, anti-Jak3, and anti-Tyk2 antibodies, and visualized by chemi-luminescent staining. There was a close association of uPAR with Jak1 and Tyk2 in both SMCs and ECs cell lysates, but not with Jak2 and Jak3.





We conclude that assemblage of uPAR, Jak1 and Tyk2 in one receptor complex indicates the functional cooperation of the uPAR and the Jak/STAT pathway as one regulator for SMC and EC migration and proliferation.

941-144 Ecto-5′-nucleotidase is Phosphorylated via Protein Kinase C in the Canine Preconditioned Myocardium

H. Funaya, M. Kitakaze, K. Yoshida[1], T. Kurihara, M. Hori. *The First Dept. of Med., Osaka Univ., Osaka, Japan,* [1] *The Dept. of Legal Med., Yamaguchi Univ., Yamaguchi, Japan*

We have reported that cardioprotection in ischemic preconditioning (IP) is attributable to activation of ecto-5′-nucleotidase (ecto-5′-NT), the enzyme responsible for adenosine production in the canine myocardium and that ecto-5′-NT is activated by protein kinase C (PKC). However, there is no direct evidence that ecto-5′-NT is the substrate for PKC in the myocardium. We examined whether PKC phosphorylates ecto-5′-NT of the canine preconditioned myocardium. In the open chest dogs, IP was produced by 4 times of 5 min coronary occlusion with a 5-min interval (n = 5). In another dogs, we administered PMA (0.5 pmol/kg/min) into the coronary artery for 4 times of 5 min with a 5-min interval (n = 5). Five min after IP and PMA procedure, we sampled myocardial tissues and measured activity of ecto-5′-NT and PKC in the membrane fraction. Furthermore, we performed the immunoblot using specific antibodies of phosphorylation protein (phosphothreonine, phosphoserine and phosphotyrosine) after immunoprecipitation of the same samples by ecto-5′-nucleotidase antibody. Activities of ecto-5′NT (71.3 ± 5.7 and 69.3 ± 4.7 vs. 42.3 ± 3.8 nmol/mg protein/min) and PKC (27.8 ± 3.5 and 26.3 ± 4.2 vs. 6.2 ± 0.9 nmol/mg protein/min) increased in the IP and PMA groups, compared with the control group, respectively. We observed the threonine-serine phosphorylation of ecto-5′-NT in the IP and PMA groups. A major band was detected by phosphothreonine and phosphoserine antibodies in the IP and PMA groups, and which was not detected by phosphotyrosin antibody in both groups. We conclude that ecto-5′-NT of the myocardium is phosphorylated and activated via protein kinase C in the ischemic preconditioning. The target enzyme that causes cardioprotection in ischemic preconditioning may be ecto-5′-NT.

941-145 Endogenous Induction of Plasminogen Activator Inhibitor in Rabbits by Angiotensin II and its Hexapeptide (3–8) Fragment

W.L. Daley, S. Plavin, D.E. Vaughan. *Brockton/West Roxbury VA Medical Center, Brigham, USA, Women's Hospital, Harvard Medical School, Boston, MA, USA, Vanderbilt University School of Medicine, Nashville, Tennessee, USA*

Plasminogen activator inhibitor (PAI-1) plays an important role in regulating the fibrinolytic system. PAI-1 is thought to be the principle physiologic inhibitor of tissue type plasminogen activator (t-PA). However, the regulation of PAI-1 is not well understood. In this study, we investigated whether the induction of PAI-1 by the renin-angiotensin system (RAS) was secondary to Angiotensin II (AngII) or its by-product the hexapeptide (3–8) fragment Angiotensin IV (AngIV). In twenty four normal New Zealand White rabbits, AngII (n = 6) and AngIV (n = 6) were infused at graded concentration (2, 4 and 8 μg/kg/min) over 180 minutes. Mean levels of PAI-1 activity increased significantly in a dose dependent manner from (4 ± 3.7 ng/mL) before AngII infusion to (26 ± 3.3 ng/mL) at the end of AngII infusion ($p < 0.00001$) and from (1.1 ± 1.02 ng/mL) before AngIV infusion to (27 ± 3.7 ng/mL) at the end of AngIV infusion ($p = < 0.00001$), whereas PAI-1 activity decreased in the control group infused with normal saline (n = 6), from (4.8 ± 1.5 ng/mL) to (1 ± 0.5 ng/mL) ($p = < 0.001$). The animals that were pretreated with amastatin (AngII receptor blocker, n = 6) prior to receiving graded infusion of AngII, exhibited the expected rise in blood pressure while the PAI-1 activity was significantly reduced (8.7 ± 2.1 ng/mL) in comparison to the infusion of AngII alone ($p = < 0.00001$).

Conclusion: These *in vivo* data strongly suggest that the increase in the circulating levels of PAI-1 induced by the renin-angiotensin system maybe secondary to both AngII and AngIV.

941-146 In Vitro Fibrillin Assembly and Biosynthetic Abnormalities of Fibrillin in the Marfan Syndrome

T. Brenn, U. Franke[1], H. Furthmayr. *Department of Pathology, University School of Medicine, Stanford, USA,* [1] *Department of Genetics, Howard Hughes Medical Institute, Stanford, USA*

The Marfan syndrome has been linked to the extracellular matrix protein fibrillin-1 (FBN1) which aggregates to form insoluble microfibrils. Mutations in the FBN1 gene result in abnormal patterns of synthesis, secretion and matrix deposition of FBN1 in dermal fibroblasts as well as reduced immunofluorescent staining of microfibrils. Groups of patients were identified by a quantitative pulse chase technique measuring FBN1, which correlate with severity and prognosis of the disease. The present study was designed to better define the biosynthesis and assembly of FBN1 using the quantitative pulse chase technique in combination with indirect immunofluorescence *in vitro*. When normal human fibroblasts were grown (1ºculture), trypsinized and maintained for an additional period (2º culture), FBN1 in the tissue culture medium could readily be separated from an intracellular and extracellular microfibrillar form. While the synthesis of FBN1 was not affected, the amount of insoluble, microfibrillar FBN1 was quite sensitive to culture conditions in the 1º culture as well as the 2º culture. Trypsinization of labeled fibroblasts under conditions used for replating of cells showed incomplete digestion of insoluble, microfibrillar FBN1 and FBN1 was also detected by immunofluorescence in fragmented and clumpy microfibrils one day after replating of the trypsinized cells, suggesting that insoluble microfibrillar material had been transferred together with the cells. Four days of culture were required for the formation of a normal microfibrillar network and longer culture times did not change the staining pattern. This suggests that preformed microfibrils act as 'nuclei' for the incorporation of newly formed FBN1. In certain MFS fibroblasts a considerable reduction and an altered pattern in microfibrillar organization is observed. Our data indicate that the pulse chase assay measures a reduction in FBN1 deposition in such fibroblasts, since fewer microfibrils are available for the deposition of a labeled FBN1 molecule during the chase period. Altered microfibrillar structures assembled in culture may also contribute to the reduction in matrix deposition of FBN1 due to the dominant negative effect, in spite of a relatively short chase period.

941-147 A Missense Mutation of the eNOS Gene in Patients with Coronary Heart Disease and Its Possible Interaction with ACE I/D Gene Polymorphism for Coronary Risk

K. Liu, H. Bai, K. Saku, T. Hiroki, K. Arakawa. *Fukuoka University School of Medicine, Fukuoka, Japan*

In coronary angiographically (CAG) proven-coronary heart disease (CHD) patients, we identified a missense mutation in exon 7 in the eNOS gene by direct DNA sequencing: a G to T transition that changed Glu^{298} (GAG) to Asp (GAT). We screened this mutation in CAG proven-CHD patients (n = 432) and normal controls (n = 207). The variant distribution of this mutation was 16.7% in patients, but only 7.6% in controls. This difference was significant ($\chi^2 = 9.413$, $p = 0.002$). No significant differences were found among patient subgroups with 1-vessel, 2-vessel and 3-vessel stenosis. However, the frequency of variants with this mutation in patients (n = 48) with restenosis after percutaneous transluminal coronary angioplasty (PTCA) is markedly higher than that in patients (n = 56) without restenosis (27.1% vs 12.5%, $\chi^2 = 4.638$, $p = 0.031$). Multiple logistic regression analysis revealed a significant association between the risk of CHD and this eNOS missense mutation ($p = 0.032$). To evaluate the possibility of an interaction between this mutation and ACE I/D polymorphism on the risk of CHD, we screened the ACE I/D genotypes. In variants with this mutation, the odds ratio for CHD associated with the ACE DD genotype was 4.71 (2.32–9.58, p = 0.039). Among patients defined as low-risk (serum apo B < 1.25 g/L, BMI < 26 kg/m^2, non-HT, non-DM, and non-smoking), this interaction is even stronger [odds ratio: 11.56 (5.15–25.91, p = 0.010)]. *Conclusions:* 1. The frequency of the $Glu^{298} \rightarrow$ Asp missense mutation of the eNOS gene was established in Japanese controls and CHD patients; 2. This mutation may be associated with an increased risk for CHD; 3. This mutation may interact with ACE I/D polymorphisms to affect the risk for CHD.

941-148 Adenoviral-Mediated Gene Therapy Induces Sustained Intrapericardial Vascular Endothelial Growth Factor Expression in Dogs: Effect on Myocardial Angiogenesis

D.F. Lazarous, M. Shou, J.A. Stiber, V. Thirumurti, E. Hodge, E.F. Unger. *NHLBI, Bethesda, MD, USA*

We examined the effects of intrapericardial delivery of a replication-deficient adenovirus carrying the cDNA for vascular endothelial growth factor (AdCMV.$VEGF_{165}$) on myocardial angiogenesis in an established dog collateral model. Ameroid constrictors were placed on the proximal left circumflex coronary artery of dogs. Ten days later, 6×10^9 pfu AdCMV.VEGF (n = 5), AdRSV.βgal (n = 4), or saline (n = 7) were delivered through an indwelling pericardial catheter. Transfection efficiency was high, with extensive βgal staining in the epi- and pericardium. Pericardial and serum VEGF levels were measured by ELISA in 6 dogs (average values shown). Collateral perfusion, assessed with radiolabeled microspheres 28 days post-Rx, was similar in all groups.